***Title***:

***Background:*** The Laurentian Great Lakes represent the largest freshwater ecosystem on Earth and supports countless human populations and their economies (Rau et al., n.d.). Of particular concern are the commercial fisheries that have been in decline since the 1940s, which have been attributed to overfishing, invasive species, chemical pollution, and declining nutrient levels (Hudson & Ziegler, 2014). Much of what we know about nutrient levels and cycling in the Great Lakes is limited to spring and summer, with few *in situ* Great Lakes studies representing winter processes (Pu et al., 2025). Recent work has brought to light the vital role that winter ecological and biogeochemical processes play in year-round conditions, with impacts that are felt in the subsequent spring and summer (Hampton et al., 2017; Özkundakci et al., 2016; Sommer et al., 2012; Wen et al., 2020). Changing winter conditions can upset normal lake processes and can have cascading effects on ecological and biogeochemical processes that can, in turn, threaten the Great Lakes’ water quality and health of biological communities (Hampton et al., 2017). It is important to understand how microbial communities will react to different environmental conditions because microbes exist at the base of many food webs and are responsible for recycling nutrients and organic matter in aquatic systems (need a citation). It is because of their trophic position and integral part of biogeochemical cycling, potential changes to those communities can be felt throughout the ecosystems they reside in (need another citation here). Microbial communities can fluctuate in their assemblages (Adams et al., 2014; Michoud et al., 2025), their metabolism (*Global Emergent Responses of Stream Microbial Metabolism to Glacier Shrinkage | Nature Geoscience*, n.d.), morphology (Morán et al., 2015), and stoichiometry (Cotner et al., 2010; Godwin & Cotner, 2015) as responses to environmental variables such as temperature, dissolved organic matter (DOM), and nutrient availability. Globally, DOM inputs into northern lakes have increased, and the consequences are relatively unknown(*Global Change Biology - 2018 - Creed - Global Change‐driven Effects on Dissolved Organic Matter Composition Implications (1).Pdf*, n.d.). The current evidence shows that increased DOM inputs are expected to change the availability of macronutrients (C, N, P) and several micronutrients (Fe, Mn, etc.) (*A Test of the Subsidy–Stability Hypothesis: The Effects of Terrestrial Carbon in Aquatic Ecosystems - Jones - 2015 - Ecology - Wiley Online Library*, n.d.), which in turn influence the stoichiometry of biological communities such as phytoplankton and bacteria. Additionally, the stoichiometry of bacterial communities has also been shown to be coupled with temperature (Phillips et al., 2017). The interplay between annual variation in winter variables (such as ice cover and water temperature), seasonal variation, and increased DOM inputs is not well understood, and we hope to provide additional insight with our research.Winter is an integral component of annual limnological processes, the better we will be able to apply effective measures to manage the Great Lakes and maintain healthy

***Goals and Hypothesis:*** The goal of my proposed research is to investigate how seasonality impacts microbial communities, with an emphasis on the transition from winter to spring. **Objective 1:** Characterize the response of microbial stoichiometry to changes in particulate organic matter in conjunction with seasonal changes in temperature **Hypothesis 1 (H1):** Oligotrophic systems will be more susceptible to temperature effects on bacterial stoichiometry, shown by an increase in P content and cell size **Objective 2:** evaluate microbial community resilience to altered nutrient concentrations and environmental conditions **Hypothesis (H2):** oligotrophic systems will have a lower functional redundancy when compared to eutrophic systems, marked by more specialized taxa when compared to eutrophic systems.

***Experimental Design:*** To investigate this, bacterial communities from Lake Superior, Huron, and Erie will be collected and transplanted into another lake via dialysis bags. Lake Superior and Huron communities will be swapped as well as Lake Huron and Lake Erie, to establish a trophic gradient. These three lakes and their watersheds also differ in morphometry, land use, and certain winter variables (such as ice cover and duration). The bacterial response to being transplanted into a different environment will be tracked throughout 48 hrs with sub-sampling taken at intervals within that time. Samples will be analyzed for particulate C, N, and P in the bacterial communities and the water inside the dialysis bag. Water samples from the host lake will also be collected for analysis at the beginning and end of the incubation period. Additionally, morphological traits will be determined via flow cytometry, and changes in community assemblage will be analyzed using 16S rRNA gene sequencing. Physical and chemical parameters such as ice cover (when applicable), dissolved oxygen, pH, and water temperature will also be measured. To measure DOM, 0.45 mm filtered water samples will be analyzed for dissolved organic carbon (DOC) and total nitrogen (TN) using a Shimadzu TOC-L, and fluorescent dissolved organic matter will be measured by emission excitation matrix spectroscopy using a Horiba Aqualog.

The large knowledge gap concerning winter limnology provides an opportunity to gather more insight into the limnological processes occurring in the Great Lakes, and how various biological communities may be impacted by winter conditions. This is especially relevant to the Great Lakes since they experience interannual variability in winter severity, such as ice on, ice duration, and ice cover. It is a well-understood concept in ecology that changes to the base of the food web can have rippling effects that impact larger trophic levels, and the ecological stoichiometry of bacterial communities provides another lens to assess ecosystem health

This is largely attributed to traditional limnological views that labeled winter as a time of inconsequential biological activity, therefore being of little importance to annual and intraseasonal lake processes (Hampton et al., 2015; Powers & Hampton, 2016; Salonen et al., 2009). Most importantly, and perhaps the underlying reason for traditional limnological views, is the logistical difficulty in winter sampling, which is impeded by hazardous conditions, expensive equipment, and a field sampling period that is concurrent with academic calendars (Block et al., 2019; Bolsenga et al., 1988).

One such variable, ice cover, is notoriously variable between years and among lakes (Fujisaki et al., 2012; *Simulating Impacts of Precipitation on Ice Cover and Surface Water Temperature Across Large Lakes - Fujisaki-Manome - 2020 - Journal of Geophysical Research: Oceans - Wiley Online Library*, n.d.).

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